Development of Immune Boosting Dietary Supplements as Alternatives to Antibiotics

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ABSTRACT

Routine use of antibiotics in animal feed at sub-therapeutic dose for growth promotion and disease prevention is suspected to be a major driving force for rapid emergence of antibiotic-resistant pathogens, which have become a serious threat to public health worldwide. To ensure public health and stable and safe supply of animal food products, alternative approaches to disease control are urgently needed. Through a comprehensive screening process, we discovered several dietary supplements to be highly effective in enhancing host innate immunity and disease resistance without triggering proinflammatory response. Of particular interest are short-chain fatty acids (SCFAs) produced naturally by intestinal commensal bacteria. We found that SCFAs strongly induces the expressions of multiple genes for endogenous antimicrobial host defense peptides (HDPs), which possess potent immunomodulatory and broad-spectrum antimicrobial activities. In addition, dietary supplementation of SCFAs reduced the titer of Salmonella enteritidis in the chicken cecum following experimental infections. We further revealed that the induction of HDP gene expression is inversely correlated with the length of the aliphatic carbon chain of free fatty acids in chicken HD11 macrophages and primary monocytes, with SCFAs being the most potent, medium-chain fatty acids moderate and long-chain fatty acids largely ineffective. Moreover, we observed a strong synergy in inducing HDP synthesis among SCFAs and between SCFAs and a botanical extract. Therefore, dietary supplementation of immune boosting SCFAs or SCFA/botanic extracts may have potential for further development as a promising antibiotic alternative approach to disease control and prevention. In addition to poultry, such an immunostimulatory approach is expected to be broadly applicable to all other animal species including humans, offering great potential of enhancing production efficiency, and food safety, while minimizing the use of antibiotics and emergence of drug-resistant pathogens.

INTRODUCTION

Widespread use of antibiotics as growth promoters is suspected to be a major source for the development of antibiotic-resistant pathogens, which have become a major public health concern worldwide. Enhancing host immunity and disease resistance by specifically boosting the synthesis of endogenous host defense peptides (HDPs) may represent a promising antibiotic-alternative strategy. HDPs have been found in nearly all forms of life and play an important role in the first line of defense. HDPs kill a broad range of microbes including bacteria, fungi, parasites, and enveloped viruses mainly through physical interaction and disruption of the membranes. It is, therefore, extremely difficult for pathogens to develop resistance. In addition to their direct antimicrobial activities, HDPs play a profound role in potentiating the immune response to infections by recruiting and activating immune cells, binding and neutralizing bacterial endotoxins, and promoting wound healing **(Fig.1)**. Because of these pleiotropic effects, it is beneficial to specifically enhance the synthesis of endogenous HDPs for disease control and prevention.

As an important source of energy, fatty acids are represented by a large group of carboxylic acids with an aliphatic hydrocarbon chain that are either saturated or unsaturated. Based on the number of carbon atoms in the aliphatic chain, fatty acids are broadly classified into three groups, namely SCFAs (≤ C5), medium-chain fatty acids (MCFAs) (C6 to C11), and long-chain fatty acids (LCFAs) (≥ C12). Butyrate, acetate, and propionate are the major species of SCFAs produced by bacterial fermentation of carbohydrates in the intestine. The concentrations of acetate, propionate, and butyrate vary in molar ratios from 48:29:23 to 70:15:15 in human feces and 33:12:6 in chicken cecal contents. Besides being the major energy source, SCFAs also possesses many other biological roles **(Fig. 2).**

Earlier studies reported that SCFAs including butyrate and propionate are capable of inducing the synthesis of LL-37, a HDP in humans, which is largely due to their histone deacetylase inhibitory activity. Inhibition of histone deacetylase is known to promote hyper-acetylation of the lysine residues in nucleosome core histones leading to a less compact chromatin and transcriptional activation of a subset of genes. Here we tested the ability of SCFAs to induce HDP gene expression and enhance chicken host immunity and disease resistance. We further compared the relative potency in HDP induction among free fatty acids of various aliphatic chain lengths (C1 to C18). Additionally, we studied possible synergistic interactions among three SCFAs including acetate, propionate, and butyrate in enhancing chicken HDP gene expression and reducing bacterial colonization. Collectively, our results suggested the potential for dietary supplementation of SCFAs as an alternative approach in disease control and prevention .

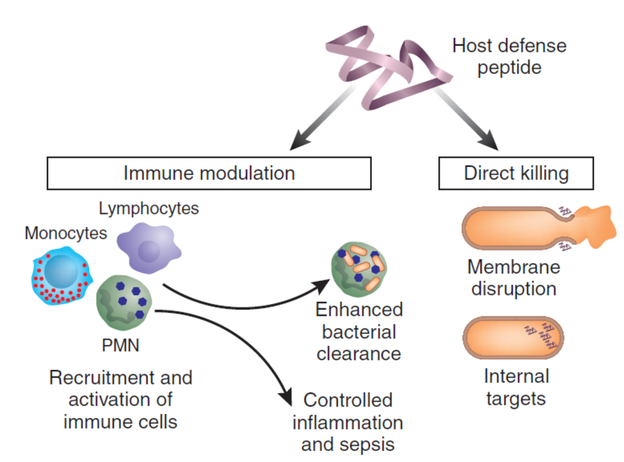


Fig. 1. Biological functions of host defense peptides (HDPs) . Besides direct microbicidal activities, HDPs play a profound role in immunomodulation (Adopted from Hancock & Sahl. 2006. Nature Biotech 24:1551).

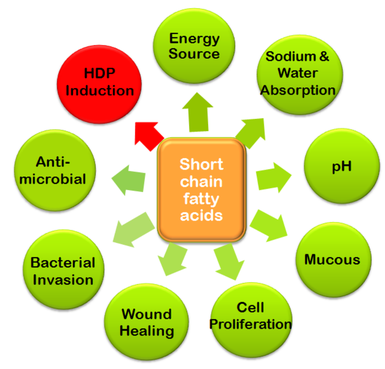


Fig. 2. Pleotropic functions of short-chain fatty acids. Besides being the major energy source, SCFAs are involved in many pathophysiological processes of the intestinal tract, including mucosal host defense, gut motility and nutrient absorption, and epithelial cell proliferation, differentiation, and apoptosis.

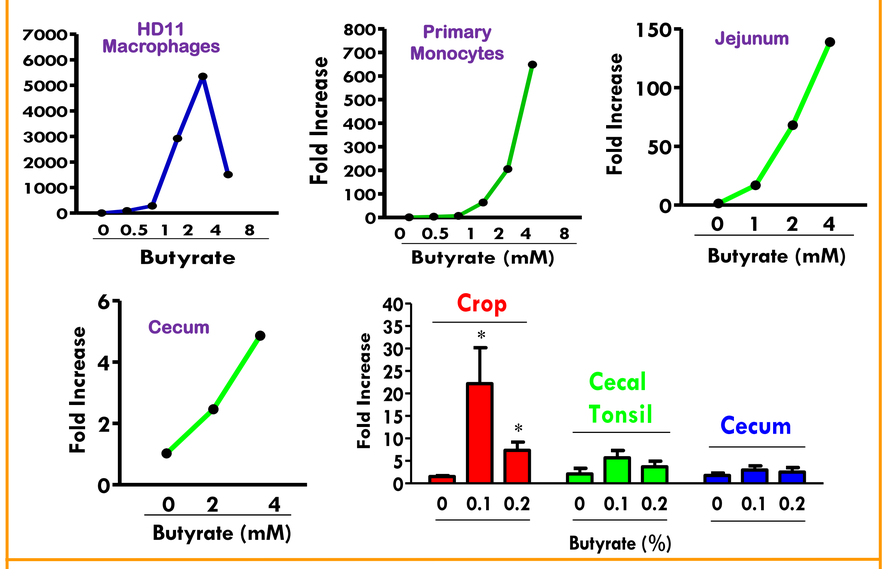


Fig. 3. In vivo and in vitro induction of the *AvBD9* gene expression by butyrate in chickens. Chicken immortalized or primary cells were stimulated with sodium butyrate for 24 h, and then subjected to real-time RT-PCR analysis. In the bottom right panel, 2-day-old broilers were fed with or without sodium butyrate for 2 days. The *AvBD9* gene expression was evaluated. Each bar represents means ± standard error of the data from 6 different chickens. *$P < 0.05$ by unpaired Student's *t*-test.

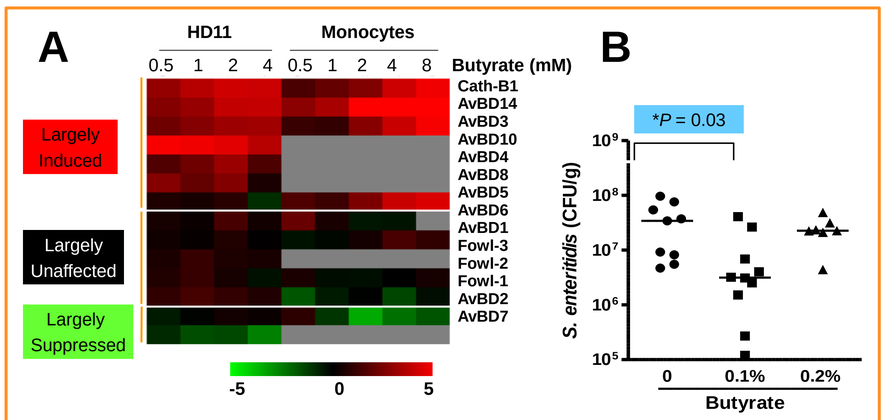


Fig. 4. Up-regulation of multiple HDPs in chicken cells by butyrate (A) and reduction of *S. enteritidis* colonization in chickens fed with butyrate (B). In ***A***, the color elements represent average $\log_2$ ratios of the fold change from 2-3 independent experiments. Red indicates up-regulation, whereas black means no induction and green down-regulation. Gray areas are an indication of no data due to extremely low expression levels of certain HDPs. In ***B***, broilers were supplemented with or without butyrate in feed for 2 days, followed by an inoculation with *S. enteritidis* phage type 13a. Butyrate supplementation was continued for another 4 days before the cecal content was collected and bacterial number enumerated. Each dot indicates the bacterial titer in a bird and the solid line represents the median value of each treatment. *$P = 0.03$ (by unpaired Student's *t*-test).

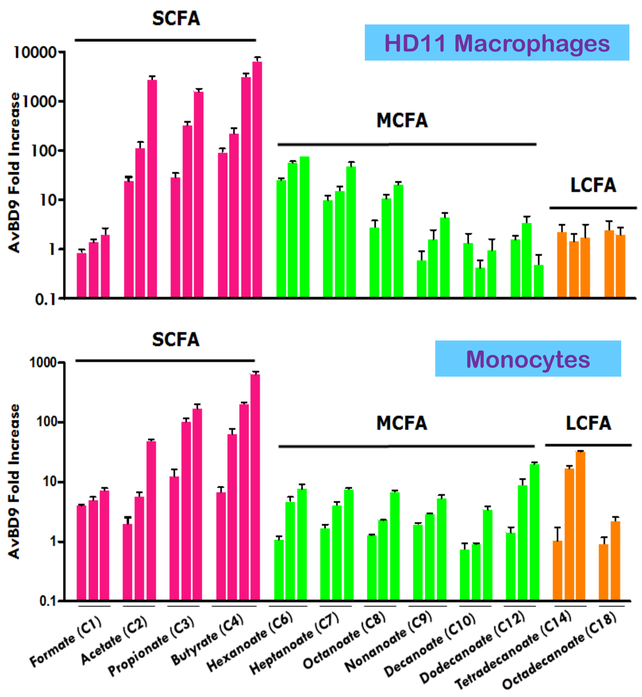


Fig. 5. Regulation of the *AvBD9* gene expression in chicken HD11 macrophage cells (A) and primary monocytes (B) by free fatty acids. HD11 and primary monocytes were stimulated with different concentrations of free fatty acids in their sodium salt form for 24 h and then subjected to real-time RT-PCR analysis.

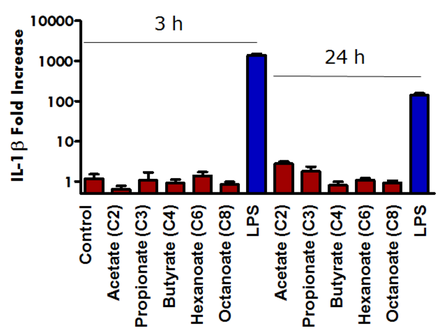


Fig. 6. No Induction of *IL-1β* in chicken HD11 cells by free fatty acids. Cells were incubated in duplicate with or without fatty acids or lipopolysaccharides (LPS) for 3 and 24 h, followed by real-time RT-PCR analysis of *IL-1β*.

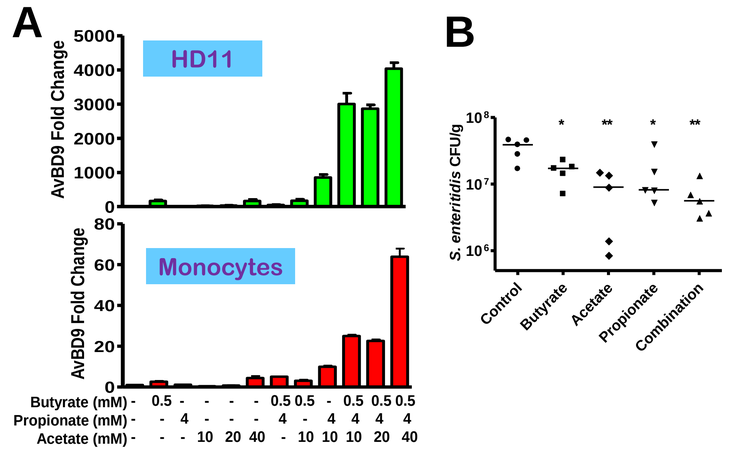


Fig. 7. SCFA-induced synergistic induction of *AvBD9* expression in chicken cells (A) and reduction of the *S. enteritidis* titer in chickens (B). In ***A***, cells were incubated with acetate, propionate, and butyrate alone or in combinations for 24 h, followed by real-time RT-PCR. In ***B***, 4-day-old broilers were supplemented with or without individual SCFAs or their combinations in water for 2 days with 5 birds per group, followed by an inoculation with *S. enteritidis* phage type 13a. SCFA supplementation was continued for another 4 days before the cecal content was collected and bacterial number enumerated. *$P < 0.05$ and **$P < 0.01$ (by unpaired Student's *t*-test).

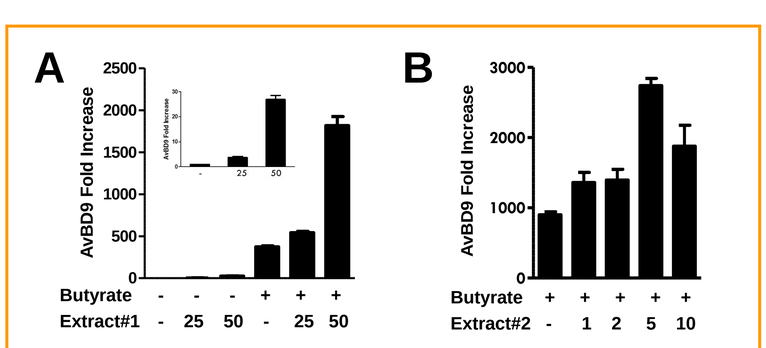
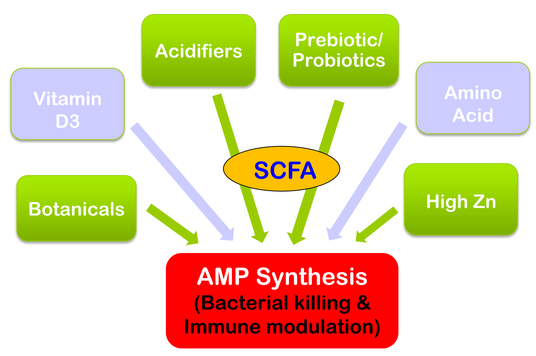


Fig. 8. Synergistic induction of *AvBD9* expression between butyrate and plant extracts in chicken mononuclear cells (A) and HD11 macrophages (B). Cells were incubated with butyrate with or without different plant extracts for 24 h, followed by real-time RT-PCR analysis.

CONCLUSIONS

- Butyrate selectively induces HDP gene expression both in vitro and in vivo and enhances pathogen clearance with a minimal impact on the proinflammatory response.
- HDP gene expression is inversely correlated with the aliphatic carbon chain length of free fatty acids, with SCFAs being most potent inducers, MCFAs moderate, and LCFAs largely ineffective;
- SCFAs induce *AvBD9* expression and reduce the *S. enteritidis* colonization in chickens in a synergistic manner;
- SCFAs and their analogs may have potential for further development as a cost-effective, antibiotic-alternative approach in disease control and prevention.



ACKNOWLEDGEMENTS

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